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CLAIMS

What is claimed is:

- 5 1. An isolated perhydrolase, wherein said perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1.
2. The perhydrolase of Claim 1, wherein said perhydrolase is *M. smegmatis* perhydrolase.
- 10 3. An isolated perhydrolase, wherein said perhydrolase is at least approximately about 35% homologous to said *M. smegmatis* perhydrolase of Claim 2.
4. The perhydrolase of Claim 2, wherein said perhydrolase comprises the
15 amino acid sequence set forth in SEQ ID NO:2.
5. An isolated perhydrolase having immunological cross-reactivity with said perhydrolase of Claim 2.
- 20 6. The perhydrolase of Claim 2, wherein said perhydrolase is at least a portion of said *M. smegmatis* perhydrolase, wherein said perhydrolase has a perhydrolysis to hydrolysis ration that is greater than 1.
7. The perhydrolase of Claim 2, wherein said perhydrolase is a structural
25 homologue of said *M. smegmatis* perhydrolase, in which the active site is homologous to at least one amino acid selected from the group consisting of S11, D192, and H195 of the *M. smegmatis* perhydrolase.

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8. An isolated perhydrolase variant having an amino acid sequence comprising at least one modification of an amino acid made at a position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2.

9. The perhydrolase variant of Claim 8, wherein said at least one modification is made at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said modified amino acid is selected from the group consisting of Cys7, Asp10, Ser11, Leu12, Thr13, Trp14, Trp16, Pro24, Thr25, Leu53, Ser54, Ala55, Thr64, Asp65, Arg67, Cys77, Thr91, Asn94, Asp95, Tyr99, Val125, Pro138, Leu140, Pro146, Pro148, Trp149, Phe150, Ile153, Phe154, Thr159, Thr186, Ile192, Ile194, and Phe196.

10. The perhydrolase variant of Claim 8, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of M1, K3, R4, I5, L6, C7, D10, S11, L12, T13, W14, W16, G15, V17, P18, V19, D21, G22, A23, P24, T25, E26, R27, F28, A29, P30, D31, V32, R33, W34, T35, G36, L38, Q40, Q41, D45, L42, G43, A44, F46, E47, V48, I49, E50, E51, G52, L53, S54, A55, R56, T57, T58, N59, I60, D61, D62, P63, T64, D65, P66, R67, L68, N69, G70, A71, S72, Y73, S76, C77, L78, A79, T80, L82, P83, L84, D85, L86, V87, N94, D95, T96, K97, Y99F100, R101, R102, P104, L105, D106, I107, A108, L109, G110, M111, S112, V113, L114, V115, T116, Q117, V118, L119, T120, S121, A122, G124, V125, G126, T127, T128, Y129, P146, P148, W149, F150, I153, F154, I194, and F196.

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11. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in peracid hydrolysis compared to the wild-type perhydrolase.

12. The perhydrolase variant of Claim 11, wherein said change in peracid hydrolysis is a decrease.

13. The perhydrolase variant of Claim 11, wherein said change in peracid hydrolysis is an increase.

14. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.1 or less, in comparison with wild-type perhydrolase.

15. The perhydrolase variant of Claim 14, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of R4, L12, G15, P18, R27, W34L38, A44, E51, G52, L53, S54, T58, R67, L68, S72, A79, T80, D85, L86, V87, N94, K97, R101, V118, L119, G124, G126, and I194.

16. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.2 or less, in comparison with wild-type perhydrolase.

17. The perhydrolase variant of Claim 16, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID

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NO:2, wherein said at least one substitution is selected from the group consisting of R4, I5, D10, L12, W14, G15, P18, V19, T25, R27, W34, L38, A44, I49, E50, E51, G52, L53, S54, A55, R56, T58, N59, D62, T64, D65, R67, L68, N69, S72, S76, C77, A79, T80, D85, L86, V87, N94, K97, R101, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, V118, L119, A122, G124, G126, T127, Y129, W149, and I194.

18. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.3 or less, in comparison with wild-type perhydrolase.

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19. The perhydrolase variant of Claim 18, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of R4, I5, D10, L12, W14, G15, L12, P18, V19, G22, A23, T25, E26, R27, W34, G36, L38, Q41, L42, G43, A44, I49, E50, E51, G52, L53, S54, A55, R56, T57, N59, T58, D62, T64, D65, R67, L68, N69, G70, S72, Y73, S76, C77, A79, T80, L82, P83, D85, L86, V87, N94, T96, K97, Y99, F100, R101, R102, P104, L109, G110, M111, L114, V118, L119, A122, G124, V125, G126, T127, Y129, W149, F154, and I194.

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20. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.4 or less, in comparison with wild-type perhydrolase.

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21. The perhydrolase variant of Claim 20, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID

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NO:2, wherein said at least one substitution is selected from the group consisting of R4, I5, L6, D10, S11, L12, W14, G15, W16, P18, V19, G22, A23, T25, E26, R27, F28, W34, T35, G36, L38, Q41, L42, G43, A44, D45, E47, I49, E50, E51, G52, L53, S54, A55, R56, T57, T58, N59, T58, I60, D62, T64, D65, R67, L68, N69, G70, S72, Y73, S76, C77, A79, T80, L82, P83, D85, L86, V87, N94, P66, T96, K97, Y99, F100, R101, R102, P104, I107, L109, G110, M111, S112, L114, V118, L119, S121, A122, G124, V125, G126, T127, Y129, W149, F150, F154, I194, and F196.

22. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.5 or less, in comparison with wild-type perhydrolase.

23. The perhydrolase variant of Claim 22, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109,

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L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

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24. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.6 or less, in comparison with wild-type perhydrolase.

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25. The perhydrolase variant of Claim 24, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of

15 A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87,

20 N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109,

25 L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194,

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K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, V125, V19, W16, Y129, Y73, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

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26. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.7 or less, in comparison with wild-type perhydrolase.

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27. The perhydrolase variant of Claim 26, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of

15 A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54,

20 A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85,

25 E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106,

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D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196, F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53, L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72, T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129, Y73, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

28. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.8 or less, in comparison with wild-type perhydrolase.

29. The perhydrolase variant of Claim 28, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y1d29, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85,

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E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196, F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53, L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72, T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129, Y99, A108, A122, A23, A29, A44, A55, A71, A79, C77, D45, D61, D65, D85, D95, E47, E51, F150, F196, F46, G110, G126, G36, G43, G52, I107, I194, I49, I5, I60, I89, L114, L42, L53, L68, L78, L84, M111, N59, N94, P146, P24, P30, P63, P66, P83, Q117, R101, R4, S112, S121, S72, T116, T120, T127, T13, T57, T96, V113, V125, V17, V19, V32, V87, W149, Y129, Y73, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

30. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 1.5 or greater, in comparison with wild-type perhydrolase.

31. The perhydrolase variant of Claim 30, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109,

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L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116,
 T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194,
 K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58,
 T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54,
 5 A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87,
 N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23,
 A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49,
 K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56,
 R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85,
 10 E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109,
 L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25,
 T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106,
 D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194,
 K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63,
 15 P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96,
 V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196,
 F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53,
 L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72,
 T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129,
 20 Y99, A108, A122, A23, A29, A44, A55, A71, A79, C77, D45, D61, D65, D85, D95,
 E47, E51, F150, F196, F46, G110, G126, G36, G43, G52, I107, I194, I49, I5, I60, I89,
 L114, L42, L53, L68, L78, L84, M111, N59, N94, P146, P24, P30, P63, P66, P83, Q117,
 R101, R4, S112, S121, S72, T116, T120, T127, T13, T57, T96, V113, V125, V17, V19,
 V32, V87, W149, Y129, and Y73, Y99, A108, A44, C7, D10, D106, D31, D61, D85,
 25 E26, E51, F100, F28, F46, G110, G22, G36, G43, G52, G70, I107, I153, I49, I5, I89, K3,
 L105, L53, L6, L78, L86, M1, N69, P104, P146, P18, P24, P30, P83, Q117, Q40, Q41,
 R102, R27, R33, R4, S121, S72, S76, T120, T128, T13, T35, T80, T96, V115, V118,

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V32V48, V87, W34, G190, V191, G193, T197, E198, A199, R202, D203, G205, V206, A209, E210, Q211, S214, and L215.

5 32. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis between about 1.2 and about 1.5, in comparison with wild-type perhydrolase.

10 33. The perhydrolase variant of Claim 32, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, C7, D106, D31, D61, D85, E26, E50, E51, F100, F150, F28, F46, G110, G126, G22, G70, I107, K3, L105, L42, L6, L78, M111, N59, N69, P104, P146, P148, P18, P30, 15 P63, Q117, Q40, Q41, R102, R27, R33, R4, S54, S76, T116, T120, T128, T64, T80, T96, V113, V115, V118, W34, and Y73.

20 34. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is at least about 1.2.

25 35. The perhydrolase variant of Claim 34, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of C7, D10, L12, G15, P18, V19, G22, T25, E26, R27, F28, A29, P30, D31, G36, Q40, Q41, L42, G43, A44, D45, F46, E47, I49, E51, L53, S54, A55, T57, D61, P63, T64, D65, P66,

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R67, L68, N69, A71, S72, Y73, S76, L78, A79, T80, L82, P83, D85, L86, D95, K97, R101, T103, P104, L105, D106, I107, L109, M111, V113, Q117, V118, S121, G124, V125, G126, T127, P148, F150, I153, F154, and F196.

5 36. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.8 or less.

 37. The perhydrolase variant of Claim 36, wherein said modification
10 comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of
A108, A122, A23, A29, A44, A55, A71, A79, C7, C77, D10, D106, D21, D45, D61,
D62, D65, D85, E26, E47, E50, E51, F100, F150, F154, F196, F28, F46, G110, G124,
15 G126, G15, G22, G36, G52, G70, I107, I153, I194, I49, I5, I60, I89, K3, K97, L105,
L109, L114, L119, L12, L38, L42, L53, L6, L68, L78, L82, L84, K86, M1, M111,
N59N94, P146, P18, P24, P30, P66, P83, Q40, Q41, R101, R102, R27, R33, R4, R56,
R67, S11, S112, S54, S72, S76, T103, T116, T120, T127, T128, T13, T25, T35, T57,
T64, T80, T96, V113, V115, V118, V125, V17, V19, V32, V48, V87, W13, W149, W16,
20 W34, Y129, Y73, and Y99.

 38. The perhydrolase variant of Claim 8, wherein said modification comprises
at least one substitution at an amino acid position equivalent to a position in *M.*
smegmatis perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2,
25 wherein said at least one substitution is selected from the group consisting of A108,
A122, A23, A29, A44, A55, A71, A79, C7, C77, D10, D106, D21, D31, D45, D61, D62,
D65, D85, E26, E47, E50, E51, F100, F150, F154F196, F28, F46, G110, G124, G126,

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G15, G22, G36, G43, G52, G70, I107, I153, I194, I49, I5, I60, I89, K3, K97, L105, L109,
L114, L119, L12, L38, L42, L53, L6, L68, L78, L82, L84, L86, M1, M111, N59, N69,
N94, P104, P146, P148, P18, P24, P30, P63, P66, P83, Q117, Q40, Q41, R101, R102,
R27, R33, R4, R56, R67, S11, S112, S121, S54, S72, S76, T103, T116, T120, T127,
5 T128, T13, T25, T35, T57, T58, T64, T80, T96, V113, V115, V118, V125, V17, V19,
V32, V48, V87, W14, W149, W16, W34, Y129, Y73, and Y99.

39. The perhydrolase variant of Claim 8, wherein said variant perhydrolase
exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase
10 perhydrolysis to wild-type perhydrolase perhydrolysis is between about 1.2 and about 2.

40. The perhydrolase variant of Claim 39, wherein said modification
comprises at least one substitution at an amino acid position equivalent to a position in
M. smegmatis perhydrolase comprising the amino acid sequence set forth in SEQ ID
15 NO:2, wherein said at least one substitution is selected from the group consisting of C7,
D10, L12, G15, P18, V19, G22, T25, E26, R27, F28, A29, P30, D31, G36, Q40, Q41,
L42, G43, A44, D45, F46, E47, I49, E51, L53, S54, A55, T57, D61, P63, T64, D65, P66,
R67, L68, N69, A71, S72, Y73, S76, L78, A79, T80, L82, P83, D85, L86, D95, K97,
R101, T103, P104, L105, D106, I107, L109, M111, V113, Q117, V118, S121, G124,
20 V125, G126, T127, P148, F150, I153, F154, F196, G190, E198, A199, R202, D203,
V206, A209, E210, Q211, and V212.

41. The perhydrolase variant of Claim 8, wherein said variant perhydrolase
25 exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase
perhydrolysis to wild-type perhydrolase perhydrolysis is between about 2 and about 2.5.

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42. The perhydrolase variant of Claim 41, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A44,
5 C7, D10, D85, D95, E26, E47, I107, L12, L42, P104, P148, S54, Q40, Q117, D203, V206, E210.

43. The perhydrolase variant of Claim 8, wherein said variant perhydrolase
10 exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 2.5 and about 3.

44. The perhydrolase variant of Claim 43, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in
15 *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A44, C7, I107, K97, L12, L78, P104, Q40, and V125.

45. The perhydrolase variant of Claim 8, wherein said variant perhydrolase
20 exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 3.0 and about 5.

46. The perhydrolase variant of Claim 45, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in
25 *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of D10, D85, L53, L78, and S54.

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47. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.1 or less.

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48. The perhydrolase variant of Claim 47, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, and W34.

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49. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.2 or less.

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50. The perhydrolase variant of Claim 49, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, and Y73.

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51. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.3 or less.

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52. The perhydrolase variant of Claim 51, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, and Y129.

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53. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.4 or less.

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54. The perhydrolase variant of Claim 53, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6,

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L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, and V87.

55. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.5 or less.

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56. The perhydrolase variant of Claim 55, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77,

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E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109, L119, L12, L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, and Y129.

57. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.6 or less.

58. The perhydrolase variant of Claim 57, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109, L119, L12, L38, L6, L68, L84, L86, M111, N59, P146, P24,

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Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, G126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115, V118, V32, V48, V87, W149, and Y73.

59. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.7 or less.

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60. The perhydrolase variant of Claim 59, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109, L119, L12, L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115,

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V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, G126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115, V118, V32, V48, V87, W149, Y73, A122, A23, A29, A71, A79, C7, D61, D62, D85, E26, E51, F100, F28, F46, G110, G126, G52, G70, I107, I49, I5, I60, I89, L109, L114, L12, L38, L68, L82, L86, M111, N59, N94, P83, R102, R33, R4, S112, S72, S76, T103, T116, T128, T25, T35, T57, T58, T64, V19, V32, V48, V87, Y129, Y73, and Y99.

61. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.8 or less.

62. The perhydrolase variant of Claim 36, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36,

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I107, I49, I5, K97, L109, L119, L12, L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, G126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115, V118, V32, V48, V87, W149, Y73, A122, A23, A29, A71, A79, C7, D61, D62, D85, E26, E51, F100, F28, F46, G110, G126, G52, G70, I107, I49, I5, I60, I89, L109, L114, L12, L38, L68, L82, L86, M111, N59, N94, P83, R102, R33, R4, S112, S72, S76, T103, T116, T128, T25, T35, T57, T58, T64, V19, V32, V48, V87, Y129, Y73, Y99, A108, A122, A29, A55, C77, D10, D106, D45, D61, D62, D65, D85, E47, E50, F100, F150, F28, F46, G110, G124, G126, G15, G36, I153, I194, I5, I60, I89, K3, K97, L105, L109, L114, L119, L38, L42, L68, L84, L86, M1, N59, P24, P30, P83, R101, R27, R4, R56, S112, S54, S76, T103, T116, T120, T127, T128, T13, T35, T64, V113, V17, V19, V32, V48, V87, Y129, Y73, and Y99.

15

63. A perhydrolase variant, wherein said variant exhibits greater perhydrolysis activity and decreased peracid hydrolysis activity as compared to wild-type perhydrolase.

20

64. The perhydrolase variant of Claim 8, wherein said perhydrolase exhibits perhydrolysis activity ratio of at least about 1.2, and peracid hydrolysis activity ratio of about 0.8 or less, as compared to wild-type perhydrolase.

25

65. The perhydrolase variant of Claim 64, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A29, A44, A55, A71, A79, C7, D10, D106, D31, D85, E26, E47, F150, F154, F196, F28,

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G124, G126, G36, G43, I153, L109, L42, L53, L109, L42, L53, L68, L82, L86, M111, N69, P104, P148, P18, P63, P66, P83, Q117, Q40, R101, R67, S54, S121, S72, S76, T25, T64, V115, and V19.

5 66. The perhydrolase variant of Claim 8, wherein said perhydrolase exhibits perhydrolysis activity ratio of at least about 1.2, a peracid hydrolysis activity ratio of about 0.8 or less, and a protein concentration ratio of at least 0.5, as compared to wild-type perhydrolase.

10 67. The perhydrolase variant of Claim 66, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A29, A44, A71, A79, C7, D85, E26, E47, E51, F150, F154, F196, F28, G124, G126, G36,
15 I153, L109, L12, L53, L68, L82, M111, N69, P104, P148, P18, P63, P66, P83, Q117, Q40, R101, R67, S121, S54, S72, S76, T25, T64, V125, and V19.

 68. A perhydrolase variant wherein said variant perhydrolase exhibits an increase in expression of said perhydrolase variant, as compared to the expression of
20 wild-type perhydrolase.

 69. The perhydrolase variant of Claim 68, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID
25 NO:2, wherein said at least one substitution is selected from the group consisting of A2, I5, C7, F8, S11, L12, T13, W14, W16, V17, P18, V19, E20, G22, A23, P24, T25, A29, P30, V32, T35, G36, V37, A39, F46, E47, S54, A55, R56, T58, I60, D61, D62, P63, T64,

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P66, R67, L68, N69, G70, S72, Y73, L74, P75, S76, C77, L78, A79, T80, L82, P83, L84, L86, I89, T93, T96, K97, A98, Y99, F100, R101, R102, T103, P104, L105, D106, I107, A108, L109, G110, S112, V113, L114, V115, T116, Q117, V118, L119, T120, S121, A122, G124, V125, G126, T127, T128, Y129, P130, P132, K133, L135, V136, S138,
5 P141, L142, A143, M145, H147, W149, F150, Q151, I153, G157, Q159, T161, T162, L164, A165, R166, V167, Y168, A170, L171, A172, M175, K176, P178, A182, G183, S184, V185, I186, T188, I194, F196, V191, N201, L208, A209, Q211, Q213, S214, L215, and L216.

10 70. An isolated protein comprising a homolog of *M. smegmatis* perhydrolase, wherein said homolog is a protein within the SGNH-hydrolase family of proteins.

71. An isolated protein having at least about 35% identity with the amino acid sequence of *M. smegmatis* perhydrolase, in which the protein comprises at least three
15 residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195.

72. An isolated protein having at least about 38% identity with the amino acid sequence of *M. smegmatis* perhydrolase, wherein said protein exhibits perhydrolysis
20 activity.

73. A homolog of *M. smegmatis* perhydrolase, wherein said homolog is a perhydrolase comprising at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT.
25

74. The homolog of Claim 72, wherein said homolog exhibits perhydrolysis.

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75. The homolog of Claim 72, wherein said homolog exhibits a perhydrolysis to hydrolysis ratio that is great than about 1.

5 76. The homolog of Claim 72, wherein said homolog is immunologically cross-reactive with antibodies raised against said *M. smegmatis* perhydrolase.

77. The homolog of Claim 72, wherein antibodies raised against said homolog cross-react with said *M. smegmatis* perhydrolase.

10 78. An isolated protein having at least about 35% identity with the amino acid sequence of at least one *M. smegmatis* perhydrolase homolog, wherein said protein exhibits perhydrolysis activity.

15 79. An isolated protein having perhydrolase activity, wherein said protein is in the form of a multimer in solution.

80. The isolated protein of Claim 79, wherein said protein is a perhydrolase that comprises a dimer.

20 81. The isolated protein of Claim 79, wherein said protein is a perhydrolase that comprises an octamer.

25 82. An isolated protein having perhydrolase activity, wherein said protein is in the form of a multimer in solution and said protein is selected from the group consisting of *M. smegmatis* perhydrolase, *M. smegmatis* perhydrolase homologs, and *M. smegmatis* perhydrolase variants.

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83. The isolated protein of Claim 79, wherein said protein is selected from the group consisting of modified serine hydrolases and modified cysteine hydrolases, wherein said modified serine hydrolases or said modified cysteine hydrolases comprise increased perhydrolase activity as compared to unmodified serine hydrolases or unmodified
5 cysteine hydrolases

84. An isolated protein having perhydrolase activity, wherein said protein comprises at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT.
10

85. The isolated protein of Claim 84, wherein said protein is obtained from a member of the *Rhizobiales*.

86. The isolated protein of Claim 84, wherein said protein is obtained from a
15 member of the genus *Mycobacterium*.

87. An isolated gene identified using at least one primer selected from the group consisting of SEQ ID NOS:21-69.

20 88. A method for identifying a perhydrolase, comprising the steps of:
a) identifying source of said perhydrolase;
b) analyzing said source to identify sequences comprising at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT;
25 c) expressing said sequences identified in step b) to produce said perhydrolase; and
d) testing said perhydrolase for perhydrolysis activity.

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89. The method of Claim 88, wherein said analyzing step is an amplification step wherein the primer sequences set forth in SEQ ID NOS:21-69 are used to amplifying said sequences comprising at least one motif selected from the group consisting of
5 GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT.

90. The method of Claim 88, wherein said source is selected from the group consisting of environmental sources and metagenomic sources.

10 91. The protein identified using the method of Claim 88.

92. An isolated nucleic acid sequence encoding the protein of Claim 91.

15 93. The method of Claim 88, wherein said protein exhibits a perhydrolysis to hydrolysis ratio that is greater than about 1.

94. The method of Claim 88, wherein said protein exhibits a perhydrolysis activity that is at least about 0.2, compared to the perhydrolysis activity exhibited by *M. smegmatis* perhydrolase.

20

95. The method of Claim 88, wherein said protein comprises at least three residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195.

25

96. The method of Claim 88, wherein said analyzing step comprises searching at least one amino acid database.

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97. The method of Claim 88, wherein said analyzing step comprises searching at least one nucleic acid database to identify nucleic acid sequences encoding the amino acid sequences of said perhydrolase.

5 98. The method of Claim 96, wherein said source is selected from the group consisting of environmental sources and metagenomic sources.

99. An isolated nucleic acid sequence encoding the protein of Claim 96.

10 100. An isolated nucleic acid sequence identified using the method of Claim 96.

101. The method of Claim 96, wherein said protein exhibits a perhydrolysis to hydrolysis ratio that is greater than about 1.

15

102. The method of Claim 96, wherein said protein exhibits a perhydrolysis activity that is at least about 0.2, compared to the perhydrolysis activity exhibited by *M. smegmatis* perhydrolase.

20 103. The method of Claim 96, wherein said protein comprises at least three residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195, as set forth in SEQ ID NO:2.

25 104. A variant perhydrolase of Claim 1, wherein said variant perhydrolase has an altered substrate specificity as compared to wild-type *M. smegmatis* perhydrolase.

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105. The variant perhydrolase of Claim 104, wherein said variant has altered para nitrophenyl caproate (PNC) activity, as compared to wild-type *M. smegmatis* perhydrolase.

5 106. A variant perhydrolase of Claim 1, wherein said variant perhydrolase has an altered pI as compared to wild-type *M. smegmatis* perhydrolase.

107. The variant perhydrolase of Claim 106, wherein said variant perhydrolase comprises at least one positively charged mutation.

10

108. The variant perhydrolase of Claim 106, wherein said variant perhydrolase comprises at least one negatively charged mutation.

109. A variant perhydrolase of Claim 1, wherein said variant has increased
15 stability, as compared to wild-type *M. smegmatis* perhydrolase.

110. The variant perhydrolase of Claim 109, wherein said stability is selected from the group consisting of thermostability, enzymatic stability, and chemical stability.

20 111. The perhydrolase of Claim 1, wherein said variant exhibits at least one altered surface property.

112. The perhydrolase of Claim 111, wherein said variant comprises mutations
at least one substitution at sites selected from the group consisting of the residues set
25 forth in Table 15-1.

113. The perhydrolase of Claim 1, wherein said perhydrolase is a variant

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perhydrolase having at least one improved property as compared to wild-type perhydrolase.

5 114. An expression vector comprising a polynucleotide sequence encoding the perhydrolase variant of Claim 113.

115. A host cell comprising said expression vector of Claim 114.

10 116. The host cell of Claim 115, wherein said host is selected from the group consisting of *Bacillus* sp., *Streptomyces* sp., *Escherichia*, and *Pantoea* sp.

117. A perhydrolase produced by said host cell of Claim 116.

15 118. A composition comprising at least a portion of the isolated perhydrolase of Claim 1.

119. The composition of Claim 118, wherein said perhydrolase comprises the amino acid sequence set forth in SEQ ID NO:2.

20 120. The composition of Claim 118, wherein said perhydrolase is encoded by the polynucleotide sequence set forth in SEQ ID NO:1.

25 121. The polynucleotide sequence of Claim 1218, wherein said sequence comprises at least a portion of SEQ ID NO:1.

122. An expression vector comprising the polynucleotide sequence of Claim 121.

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123. A host cell comprising said expression vector of Claim 122.
124. The host cell of Claim 123, wherein said host is selected from the group
5 consisting of *Bacillus* sp., *Streptomyces* sp., *Escherichia*, and *Pantoea* sp.
125. A perhydrolase produced by said host cell of Claim 124.
126. A variant perhydrolase, wherein said perhydrolase comprises at least one
10 substitution corresponding to the amino acid positions in SEQ ID NO:2, and wherein said
variant perhydrolase has better performance in at least one property, compared to wild-
type *M. smegmatis* perhydrolase.
127. An isolated polynucleotide comprising a nucleotide sequence (i) having at
15 least about 70% identity to SEQ ID NO:1, or (ii) being capable of hybridizing to a probe
derived from the nucleotide sequence set forth in SEQ ID NO:1, under conditions of
intermediate to high stringency, or (iii) being complementary to the nucleotide sequence
set forth in SEQ ID NO:1.
- 20 128. A vector comprising the polynucleotide of Claim 127.
129. A host cell transformed with the vector of Claim 128.
130. A polynucleotide comprising a sequence complementary to at least a
25 portion of the sequence set forth in SEQ ID NO:1.
131. A method of producing an enzyme having perhydrolase activity,

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comprising:

- (a) transforming a host cell with an expression vector comprising a polynucleotide having at least 70% sequence identity to SEQ ID NO:1;
- (b) cultivating said transformed host cell under conditions suitable for said host cell to produce said perhydrolase; and
- (c) recovering said perhydrolase.

132. The method of Claim 131, wherein said host cell is selected from the group consisting of *Streptomyces*, *Pantoea*, *Escherichia*, and *Bacillus* species.

133. A probe comprising a 4 to 150 polynucleotide sequence substantially identical to a corresponding fragment of SEQ ID NO:1, wherein said probe is used to detect a nucleic acid sequence coding for an enzyme having perhydrolase activity.

134. A cleaning composition comprising:

- a) at least 0.0001 weight percent of said perhydrolase of Claim 1;
- b) a molecule comprising an ester moiety; and
- c) optionally, an adjunct ingredient.

135. A cleaning composition comprising:

- a) at least 0.0001 weight percent of said perhydrolase of Claim 1;
- b) a material selected from the group consisting of a peroxygen source, hydrogen peroxide and mixtures thereof, said peroxygen source being selected from the group consisting of:
 - i. a per-salt;
 - ii. an organic peroxyacid;
 - iii. urea hydrogen peroxide;

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- iv. a carbohydrate and carbohydrate oxidase mixture, and
- v. mixtures thereof;
- c) from about 0.01 to about 50 weight percent of a molecule comprising an ester moiety; and
- 5 d) optionally, an adjunct ingredient.

136. A cleaning composition comprising:
- a) from about 0.0001 to about 1 weight percent of said perhydrolase of Claim 8;
 - 10 b) a material selected from the group consisting of a peroxygen source, hydrogen peroxide and mixtures thereof, said peroxygen source being selected from the group consisting of:
 - vi. a per-salt
 - vii. an organic peroxyacid;
 - 15 viii. urea hydrogen peroxide;
 - ix. a carbohydrate and carbohydrate oxidase mixture, and
 - x. mixtures thereof;
 - c) from about 0.01 to about 50 weight percent of a molecule comprising an ester moiety
 - 20 d) optionally, an adjunct ingredient.

137. A cleaning composition according to Claim 136, said composition comprising an adjunct ingredient.

- 25 138. A cleaning composition according to Claim 137, wherein said adjunct ingredient is selected from the group consisting of surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, enzymes, and enzyme

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stabilizers, catalytic materials, bleach activators, bleach boosters, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids, pigments and mixtures thereof.

5

139. A cleaning composition according to Claim 137, wherein:

a) said perhydrolase exhibits a perhydrolysis to hydrolysis molar ratio that is greater than about 0.1;

10

b) said per-salt is selected from the group consisting of alkalimetal perborate, alkalimetal percarbonate, alkalimetal perphosphates, alkalimetal persulphates and mixtures thereof;

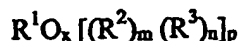
c) said carbohydrate is selected from the group consisting of mono-carbohydrates, di-carbohydrates, tri-carbohydrates, oligo-carbohydrates and mixtures thereof;

15

d) said carbohydrate oxidase is selected from the group consisting of aldose oxidase (IUPAC classification EC1.1.3.9), galactose oxidase (IUPAC classification EC1.1.3.9), cellobiose oxidase (IUPAC classification EC1.1.3.25), pyranose oxidase (IUPAC classification EC1.1.3.10), sorbose oxidase (IUPAC classification EC1.1.3.11) hexose oxidase (IUPAC classification EC1.1.3.5).
glucose oxidase (IUPAC classification EC1.1.3.4) and mixtures thereof; and

20

e) said molecule comprising an ester moiety has the formula:



25

(i) wherein R^1 is a moiety selected from the group consisting of H, substituted or unsubstituted alkyl, heteroalkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and

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heteroaryl;

- (ii) each R^2 is an alkoxylate moiety;
- (iii) R^3 is an ester-forming moiety having the formula:
 R^4CO- wherein R^4 is H, alkyl, alkenyl, alkynyl, aryl,
 5 alkylaryl, alkylheteroaryl, and heteroaryl;
- (iv) x is 1 when R^1 is H; when R^1 is not H, x is an integer that is
 equal to or less than the number of carbons in R^1 ;
- (v) p is an integer that is equal to or less than x ;
- (vi) m is an integer from 0 to 50; and
- 10 (vii) n is at least 1

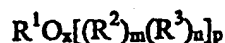
140. The cleaning composition of Claim 139, wherein:

- a) R^1 is an C_2 - C_{32} substituted or unsubstituted alkyl or heteroalkyl
 moiety;
- 15 b) each R^2 is independently an ethoxylate or propoxylate moiety; and
- c) m is an integer from 1 to 12.

141. The cleaning composition of Claim 140, wherein R^3 is an ester-forming
 moiety having the formula: R^4CO- wherein R^4 is:

- 20 a) a substituted or unsubstituted alkyl, alkenyl or alkynyl moiety
 comprising from 1 to 22 carbon atoms; or
- b) a substituted or unsubstituted aryl, alkylaryl, alkylheteroaryl or
 heteroaryl moiety comprising from 4 to 22 carbon atoms.

5 142. The cleaning composition of Claim 137, wherein the molecule comprising
 the ester moiety has the formula:



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wherein:

- 5 a) R^1 is H or a moiety that comprises a primary, secondary, tertiary or quaternary amine moiety, said R^1 moiety that comprises an amine moiety being selected from the group consisting of substituted or unsubstituted alkyl, heteroalkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl;
- b) each R^2 is an alkoxyate moiety;
- 10 c) R^3 is an ester-forming moiety having the formula:
 R^4CO- wherein R^4 may be H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl;
- d) x is 1 when R^1 is H; when R^1 is not H, x is an integer that is equal to or less than the number of carbons in R^1 ;
- e) p is an integer that is equal to or less than x
- 15 f) m is an integer from 0 to 12; and
- g) n is at least 1.

20 143. The cleaning composition of Claim 136, wherein said molecule comprising an ester moiety has a weight average molecular weight of less than 600,000 Daltons.

25 144. The cleaning composition of Claim 143, wherein said adjunct ingredient is selected from the group consisting of surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, enzymes, and enzyme stabilizers, catalytic materials, bleach activators, bleach boosters, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing

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aids, pigments and mixtures thereof.

145. A method of cleaning, said method comprising the steps of:
- 5 a) contacting a surface and/or an article comprising a fabric with the cleaning composition of Claim 136 and/or a composition comprising the cleaning composition of Claim 136; and
- b) optionally washing and/or rinsing said surface or material.
146. A method of cleaning, said method comprising the steps of:
- 10 a) contacting a surface and/or an article comprising a fabric with the cleaning composition of Claim 137 and/or a composition comprising the cleaning composition of Claim 137; and
- b) optionally washing and/or rinsing said surface or material.
147. A bleaching composition comprising the perhydrolase of Claim 1.
148. The bleaching composition of Claim 147, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases,
- 20 hemicellulases, and cellulases.
149. A bleaching composition comprising the perhydrolase variant of Claim 8.
150. The bleaching composition of Claim 149, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of
- 25 proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases,

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endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

5 151. A bleaching composition comprising the perhydrolase variant of Claim
113.

10 152. The bleaching composition of Claim 151, further comprising at least one
additional enzymes or enzyme derivatives selected from the group consisting of
proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases,
endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall
degrading enzymes, hemicellulases, and cellulases.

15 153. A bleaching composition comprising the perhydrolase variant of Claim
132.

 154. The bleaching composition of Claim 153, further comprising at least one
additional enzymes or enzyme derivatives selected from the group consisting of
proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases,
endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall
20 degrading enzymes, hemicellulases, and cellulases.

 155. A bleaching composition comprising the perhydrolase of Claim 3.

25 156. The bleaching composition of Claim 155, further comprising at least one
additional enzymes or enzyme derivatives selected from the group consisting of
proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases,
endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall

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degrading enzymes, hemicellulases, and cellulases.

157. A disinfecting composition comprising the perhydrolase of Claim 1.

5 158. The disinfecting composition of Claim 157, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

10

 159. A disinfecting composition comprising the perhydrolase variant of Claim 8.

 160. The disinfecting composition of Claim 159, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

15

20 161. A disinfecting composition comprising the perhydrolase variant of Claim 113.

 162. The disinfecting composition of Claim 161, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

25

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163. A disinfecting composition comprising the perhydrolase variant of Claim 132.

5 164. The disinfecting composition of Claim 163, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

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165. A disinfecting composition comprising the perhydrolase variant of Claim 3.

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166. The disinfecting composition of Claim 165, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

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